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Mark J. FitzGerald
Nixon Peabody LLP
100 Summer Street
Boston, MA 02110-2131

EXAMINER	
BERTAGNA, ANGELA MARIE	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/600,201	SLEPNEV, VLADIMIR I.	
	Examiner	Art Unit	
	Angela M. Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 March 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *62,63 and 65-75* is/are pending in the application.

4a) Of the above claim(s) *74 and 75* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *62,63 and 65-73* is/are rejected.

7)☒ Claim(s) *62* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of the Application

1. Applicant's response filed on March 16, 2010 is acknowledged. Claims 62, 63, and 65-75 are currently pending. In the response, claims 62, 63, 66-68, and 70 were amended, and claims 18, 19, 21-35, and 37-49 were canceled. Claims 74 and 75 remain withdrawn from consideration as being drawn to a non-elected invention.

The following objections and rejections have been withdrawn as being obviated by Applicant's amendment: (1) the objections to claims 18, 22-24, 34, 38-40, 62, 63, and 66-68, (2) the rejection of claim 70 under 35 U.S.C. 112, second paragraph, (3) the rejection of claims 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42, and 43 under 35 U.S.C. 102(e) as being anticipated by Chen, (4) the rejection of claims 23-25 and 39-41 under 35 U.S.C. 103(a) as being unpatentable over Chen in view of Woolley, and (5) the rejection of claims 28-33 and 44-49 under 35 U.S.C. 103(a) as being unpatentable over Chen in view of Nolan. Accordingly, Applicant's arguments filed on March 16, 2010 regarding these rejections and objections have been considered, but they are moot as the rejections and objections have been withdrawn.

Applicant's arguments filed on March 16, 2010 regarding the rejection of claims 62, 63, 65, 66, and 69-73 under 35 U.S.C. 103(a) as being unpatentable over Chen in view of Nolan and the rejection of claims 67 and 68 under 35 U.S.C. 103(a) as being unpatentable over Chen in view of Nolan and further in view of Woolley have been fully considered and were persuasive in part. As discussed in greater detail below, these rejections have been withdrawn. However, upon further consideration, new grounds of rejection have been made under 35 U.S.C. 103(a) citing the previously cited Chen, Nolan, and Woolley references and also the new references Li

et al. (Nucleic Acids Research (1991) 19(11): 3139-3141) and Rudi et al. (US 2006/0035222 A1). Accordingly, the finality of the Office Action mailed on September 16, 2009 has been withdrawn. This Office Action is **NON-FINAL**.

Claim Objections

2. Claim 62 is objected to because of the following informalities: The recitation "products is" in step (a) is grammatically incorrect.

Appropriate correction is required.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 62, 63, 65, 66, and 69-72 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chen et al. (US 2003/0096277 A1; cited previously) in view of **either** Li et al. (Nucleic Acids Research (1991) 19(11): 3139-3141; newly cited) **or** Rudi et al. (US 2006/0035222 A1; newly cited).

These claims are drawn to an amplification-based method of determining the identities of the nucleotides at a set of known polymorphic sites that comprises performing a first round of amplification using tagged allele-specific amplification primers, using a heat-labile exonuclease to digest allele-specific amplification primers that have not undergone polymerase-mediated

extension, and performing a second round of amplification using primers that are identical to the tag sequences.

Regarding claim 62, Chen teaches a method of determining the identities of single nucleotides at a group of known polymorphic sites, comprising:

I) providing a nucleic acid sample comprising the group of known polymorphic sites (see paragraphs 72 and 105, for example),

II) separating the strands of the nucleic acid sample and re-annealing in the presence of (see paragraphs 72 and 105; see also paragraphs 19, 27, 31-52, 55, 57, 68-72, 87-89, 103-105, Table 1, and Figures 1-2):

(a) a set of first oligonucleotide primers comprising a 3' region that hybridizes to a sequence at a known distance upstream of a known polymorphic site in the group of known polymorphic sites, wherein each member of the first set of oligonucleotide primers comprises a common sequence tag located 5' of the 3' region, and wherein the members of the first set of primers is selected such that a plurality of distinctly sized amplification products is generated for each polymorphic site in the group of known polymorphic sites (see above, and in particular, paragraphs 55 and 57 and Figure 2, where the set of reverse primers having a common sequence tag is taught. As noted above, the designations upstream, downstream, forward, and reverse are arbitrary depending on the choice of the reference strand) and

(b) a set of second oligonucleotide primers, each member of the set comprising: (i) a region that hybridizes 3' of and adjacent to a polymorphic site in the group of polymorphic sites, (ii) a variable 3' terminal nucleotide, wherein, when the member is

hybridized to the known sequence, the 3' terminal nucleotide is opposite the polymorphic site, and wherein, if and only if the 3' terminal nucleotide is complementary to the nucleotide at the polymorphic site, the 3' terminal nucleotide base pairs with the nucleotide at the polymorphic site, and (iii) a tag sequence that corresponds to the variable 3'-terminal nucleotide of (ii) with the tag sequence located 5' of region (i) on the member (see above, and in particular, Figure 2, paragraphs 31-52 and Table 1),

III) contacting the annealed oligonucleotides resulting from step (II) with a nucleic acid polymerase under conditions that permit the extension of an annealed oligonucleotide such that extension products are generated, wherein the primer extension product from the first oligonucleotide primer, when separated from its complement, can serve as a template for the synthesis of the extension product of a member of the set of second oligonucleotide primers and vice versa (see above, and in particular, paragraphs 31-52, 55, 57, 72, and 105),

IV) repeating the strand separating and contacting steps (*i.e.* steps (II) and (III)) twice, such that a population of nucleic acid molecules is generated that comprises both a sequence identical to or complementary to the first oligonucleotide and a sequence identical to or complementary to one of the members of the second set of oligonucleotides (see paragraphs 72 and 105, for example),

V) subjecting the population of nucleic acid molecules to a multiplex amplification regimen, wherein the amplification regimen is performed using an upstream amplification primer comprising the common sequence tag comprised by the first oligonucleotide primers and a set of distinguishably labeled downstream amplification primers comprising a tag comprised by a member of the set of second oligonucleotide primers and a distinguishable label (see paragraphs

72 and 105; see also paragraphs 19, 27, 31-52, 55, 57, 68-72, 87-89, 103-105, Table 1, and Figures 1-2),

VI) separating a plurality of distinctly sized amplification produced by size and/or charge (see paragraphs 62, 63, 75, and 105, for example), and

VII) detecting the incorporation of at least one distinguishable label in a plurality of distinctly sized amplification products, thereby determining the identity of the nucleotides present at each member of the set of known polymorphic sites, wherein the same set of distinguishably labeled primers determines the identities of the nucleotides at all members of the set of target polymorphic sites (see, for example, paragraphs 27, 49-51, 55, 57, 62-63, 75, and 105 and also Table 1).

Regarding claim 63, Chen teaches that the distinguishable label is a fluorescent label (see, for example, paragraphs 58 and 63 and also Table 1).

Regarding claim 65, Chen teaches that the separation method is capillary electrophoresis (paragraphs 62-63, for example).

Regarding claim 66, the amplification regimen conducted in the method of Chen comprises at least two amplification reaction cycles, with each cycle comprising the steps of: 1) nucleic acid strand separation; 2) oligonucleotide primer annealing; and 3) polymerase-mediated extension of annealed primers (see, for example, paragraph 72).

Regarding claim 69, Chen teaches a modular apparatus comprising a thermal cycling device, a sampling device, a capillary electrophoresis device, and a fluorescence detector (see, for example, paragraphs 72-75). It is noted that neither the claims nor an explicit definition in

the specification requires the elements of the modular apparatus to be physically connected to one another.

Regarding claim 70, Chen teaches that the tag sequence has a length within the claimed range (see Table 1).

Regarding claim 72, Chen teaches that the region that hybridizes 3' of and adjacent to the polymorphic site comprises 10-30 nucleotides (see Table 1).

Chen does not teach digesting unextended tagged allele-specific primers using a heat-labile exonuclease before conducting the second amplification step as required by claim 62. Chen also does not specify the length of the 3' region that hybridizes at a known distance upstream of the known polymorphic site, and therefore, does not meet the requirement in claim 71 for the length to comprise 10-30 nucleotides.

Li teaches that Exonuclease VII, which is a heat-labile exonuclease, can be used to "minimize the effect of primers left over from previous steps on subsequent reactions in which different primers are used" (page 3139, column 1). Li teaches examples that comprise treating PCR products with Exonuclease VII to degrade unextended primers, thermally inactivating the exonuclease, and amplifying the resulting reaction mixture with newly added primers (see page 3139, column 2 – page 3141, column 1). Li concludes by stating that Exonuclease VII-mediated primer degradation is rapid, amenable to automation, easy to perform, and specific (page 3139, column 1 and page 3141, column 1).

Rudi teaches a multi-step nucleic acid amplification method that comprises conducting a first amplification reaction using bipartite primers having a 3' target-specific portion and a 5' non-complementary sequence tag, digesting the unextended bipartite primers using a heat-labile

exonuclease, such as Exonuclease I, thermally inactivating the exonuclease, and conducting a second amplification reaction using primers that are identical to the 5' non-complementary sequence tag as primers (see abstract, Figure 1, and paragraphs 12-22, 27, and 76-83). Rudi teaches that including the exonuclease digestion step allows the multiplex amplification method to be quantitative in nature (paragraph 33 and 90).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to apply the teachings of either Li or Rudi to the method taught by Chen. Since Li and Rudi taught that exonuclease-mediated degradation of unextended primers between the different amplification steps of a multi-step amplification process was useful to "minimize the effect of primers left over from previous steps on subsequent reactions in which different primers are used" (see page 3139 of Li cited above) or to "maintain the quantitative nature of a multi-step multiplex nucleic acid amplification method" (see paragraphs 33 and 90 or Rudi cited above), the ordinary artisan would have been motivated to conduct an exonuclease-mediated digestion step between the two amplification reactions in the method of Chen in order to obtain the benefits described by Li or Rudi. Finally, regarding claim 71, it would have been *prima facie* obvious for one of ordinary skill in the art to conduct routine experimentation to determine the optimal length of the 3' region that hybridizes at a known distance upstream of the known polymorphic site. An ordinary artisan would have recognized from the teachings of Chen that the length of this region was a results-effective variable, and therefore, would have been motivated to conduct routine experimentation to determine the optimal length of this sequence in order to maximize amplification efficiency, for example, with a reasonable expectation of success. It is noted that no evidence has been presented to suggest that unexpected results are associated with the

claimed length range. Thus, the methods of claims 62, 63, 65, 66, and 69-72 are *prima facie* obvious over **either** Chen in view of Li **or** Chen in view of Rudi.

5. Claims 67 and 68 are rejected under 35 U.S.C. 103(a) as being unpatentable over **either** Chen et al. (US 2003/0096277 A1; cited previously) in view of Li et al. (Nucleic Acids Research (1991) 19(11): 3139-3141; newly cited) and further in view of Woolley et al. (Analytical Chemistry (1996) 68: 4081-4086; cited previously) **or** Chen et al. (US 2003/0096277 A1; cited previously) in view of Rudi et al. (US 2006/0035222 A1; newly cited) and further in view of Woolley et al. (Analytical Chemistry (1996) 68: 4081-4086; cited previously).

The combined teachings of either Chen and Li or Chen and Rudi render obvious the methods of claims 62, 63, 65, 66, and 69-72, as discussed above.

Regarding claims 67 and 68, Chen teaches a method of detecting point mutations using capillary electrophoresis and laser-induced fluorescence detection (see paragraphs 62-63, for example).

However, neither combination of references teaches analyzing an aliquot of the PCR after each reaction cycle by capillary electrophoresis as required by claims 67 and 68.

Woolley teaches a method for conducting PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device (see abstract and pages 4082-4084).

Regarding claims 67 and 68, Woolley teaches taking an aliquot after 15, 20, 25, and 30 cycles of a PCR reaction and analyzing the amount of accumulating product by capillary electrophoresis with fluorescence detection to obtain real-time monitoring of product accumulation (see page 4085 and also Figure 5). Woolley also teaches that the disclosed

integrated PCR-CE microdevices permit very rapid thermal cycling and electrophoretic separation, are suitable for use in virtually any other amplification method, permit less expensive real-time monitoring of product accumulation, and eliminate the need for sample handling between the amplification and capillary electrophoresis steps, thereby simplifying the process, eliminating pipetting errors, and reducing contamination opportunities (page 4086, column 2).

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of invention to utilize the integrated PCR-CE microdevice taught by Woolley when practicing the method resulting from the combined teachings of either Chen and Li or Chen and Rudi. An ordinary artisan would have been motivated to do so, since Woolley taught that the integrated PCR-CE microdevices permit very rapid thermal cycling and electrophoretic separation, are suitable for use in virtually any other amplification method, permit less expensive real-time monitoring of product accumulation, and eliminate the need for sample handling between the amplification and capillary electrophoresis steps, thereby simplifying the process, eliminating pipetting errors, and reducing contamination opportunities (page 4086, column 2). An ordinary artisan also would have been motivated to monitor aliquots of the amplification reaction mixture in order to monitor product accumulation in real-time as suggested by Woolley (see page 4085 & Figure 5). Finally, regarding claim 68, an ordinary artisan would have recognized from the teachings of Woolley that the number of aliquots analyzed during an amplification reaction was a results-effective variable that should be optimized by routine experimentation. As noted in MPEP 2144.05, it is *prima facie* obvious to optimize results-effective variables using routine experimentation in the absence of unexpected results. In this case, an ordinary artisan would have recognized that analyzing more aliquots (*e.g.* after each reaction cycle) would have

improved the method resulting from the combined teachings of Chen, Li, and Woolley or Chen, Rudi, and Woolley by providing more data points for analysis and a clearer picture of the exponential and plateau phases of the amplification process. Thus, the methods of claims 67 and 68 are *prima facie* obvious in view of the combined teachings of either Chen, Li, and Woolley or Chen, Rudi, and Woolley.

6. Claim 73 is rejected under 35 U.S.C. 103(a) as being unpatentable over **either** Chen et al. (US 2003/0096277 A1; cited previously) in view of Li et al. (Nucleic Acids Research (1991) 19(11): 3139-3141; newly cited) and further in view of Nolan et al. (US 6,287,766 B1; cited previously) **or** Chen et al. (US 2003/0096277 A1; cited previously) in view of Rudi et al. (US 2006/0035222 A1; newly cited) and further in view of Nolan et al. (US 6,287,766 B1; cited previously).

The combined teachings of either Chen and Li or Chen and Rudi render obvious the methods of claims 62, 63, 65, 66, and 69-72, as discussed above.

Neither combination of references suggests using a set of downstream primers consisting of four primers, each with a different 3'-terminal nucleotide as required by claim 73.

Nolan teaches a method of identifying polymorphisms using flow cytometry. Regarding claim 73, the method taught by Nolan in Example 5 (see column 7, lines 1-63) comprises an oligonucleotide ligation assay (OLA) followed by PCR amplification with an upstream primer and a set of downstream primers that may be fluorescently labeled (column 7, lines 11-50). Nolan teaches that the downstream primers each have a different 3'terminal nucleotide (see column 7, lines 27-30) in order to identify the polymorphism in a single reaction.

It would have been *prima facie* obvious for one of ordinary skill in the art at the time of the invention to apply the teachings of Nolan to the methods resulting from the combined teachings of either Chen and Li or Chen and Rudi. An ordinary artisan would have recognized from the teachings of Nolan that not all polymorphic sites may be genotyped using only one or two allele-specific downstream primers, and therefore, would have been motivated to utilize a subset of four downstream primers, each with a different 3'terminus, in order to obtain the ability to accurately type these polymorphisms. Thus, the method of claim 73 is *prima facie* obvious in view of the combined teachings of either Chen, Nolan, and Li or Chen, Nolan, and Rudi.

Response to Arguments

7. Applicant's arguments, see page 9, last paragraph, filed on March 16, 2010, with respect to the rejection of claims 62, 63, 65, 66, and 69-73 under 35 U.S.C. 103(a) as being unpatentable over Chen in view of Nolan, have been fully considered and are persuasive. As discussed in Applicant's arguments, the ordinary artisan would not have been sufficiently motivated by the teachings of Nolan to incorporate an exonuclease digestion step in the method of Chen. Accordingly, the rejection has been withdrawn. The rejection of claims 67 and 68 under 35 U.S.C. 103(a) as being unpatentable over Chen in view of Nolan and further in view of Woolley has also been withdrawn in view of Applicant's arguments at page 9, last paragraph.

Some of Applicant's arguments filed on March 16, 2010 remain pertinent to the new grounds of rejection above, where claims 62, 63, and 65-73 have been rejected under 35 U.S.C. 103(a) based on the primary combination of either Chen and Li or Chen and Rudi. These arguments have been fully considered, but they were not persuasive.

In part (ii) of the arguments (see pages 11-12), Applicant argues that methods described in Chen include a method for avoiding mismatch extension that is fully effective, and therefore, there is no reason for the ordinary artisan to include an exonuclease digestion step. This argument was not persuasive to the extent that it applies to the new grounds of rejection presented above. As discussed above, the ordinary artisan would have been motivated to add an exonuclease-mediated primer digestion step to the method of Chen, since Li and Rudi taught that this step provided a means of eliminating the ability of unextended primers from a first amplfication step to interfere with a subsequent amplification step conducted with different primers and a means of maintaining the quantitative nature of a multiplex multi-step nucleic acid amplification method, respectively.

In part (iii) of the arguments (see pages 12-13), Applicant argues that substituting an exonuclease-mediated primer digestion step for the mismatch-extension avoidance procedure described in Chen will not reduce mismatch extension and also will change the fundamental principle upon which the method of Chen operates. This argument was not persuasive, because the rejection does not state the exonuclease-mediated primer digestion step is substituted for any other step in the method of Chen. Rather, the primer digestion step is added to the method steps contained in the multi-step amplification method described by Chen. Therefore, the further inclusion of an exonuclease-mediated primer digestion step does not chage the fundamental principle of operation of the method described in Chen, because none of steps in the method of Chen are eliminated in the method resulting from the combined teachings of the cited references. Rather, an exonuclease-mediated primer digestion step is added to the method disclosed by Chen to improve the method by eliminating the abilty of the unextended allele-specific primers to

interfere with the subsequent amplification step conducted with the secondary tag primers or to maintain the quantitative nature of the multi-stage multiplex amplification method.

In part (iv) of the arguments at pages 13-14, Applicant argues that Chen teaches conducting the primary and secondary amplification reactions in the same reaction mixture, and thereby, demonstrates that the presence of unextended primary amplification primers in the reaction mixture is not detrimental. Therefore, Applicant argues, there is no motivation to include an exonuclease-mediated primer digestion step between the primary and secondary amplification steps in the method of Chen (see page 14). This argument was not persuasive, because, as discussed above, the teachings of Li or Rudi provide motivation for the ordinary artisan to add an exonuclease-mediated primer digestion step after the first round of nucleic acid amplficiation in the method of Chen. Therefore, although, as evidenced by Chen, the multi-step nucleic acid amplification process can be conducted without an exonuclease-mediated primer digestion step, the ordinary artisan would have been motivated by the teachings of either Li or Rudi to further improve the method of Chen by including such a step.

Conclusion

8. No claims are currently allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ANGELA BERTAGNA whose telephone number is (571)272-8291. The examiner can normally be reached on M-F, 9- 5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Angela M. Bertagna/
Examiner, Art Unit 1637

/Young J Kim/
Primary Examiner, Art Unit 1637